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RAPID COMMUNICATIONS

# Monoclonal Antibodies to Anchoring Fibrils for the Diagnosis of Epidermolysis Bullosa

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**Murine monoclonal antibodies to human anchoring fibrils reacted with human and monkey cervix, tongue, esophagus, and vagina. Rat, mouse, and guinea pig tissues were negative. In 11 patients with dystrophic recessive epidermolysis bullosa there was no reaction by immunofluorescence and immunoelectron microscopy. Other forms of epidermolysis bullosa had normal reactivity.**

Recessive dystrophic epidermolysis bullosa (RDEB) varies in its severity from a mild, localized disorder to a generalized disease with mutilating sequelae [1,2]. The severe, generalized (gravis) type has a marked decrease or absence of anchoring fibrils (AF) [2], and increased collagenase activity [3]. The dominantly inherited forms of EB dystrophica have morphologically abnormal AF which may be decreased in number [4].

AF are ascribed a role in stabilizing the attachment of the dermis and epithelium in skin, cervix, buccal and lingual mucosa, esophagus, and cornea [5]. AF have a characteristic ultrastructural appearance with a central transverse-banded portion and fan-shaped ends extending toward the epidermis and dermis. They form a network below the lamina densa: one end inserts directly into the lamina densa (basal lamina), the other end extends into the dermis or may connect with adjoining AF [2]. Their biochemical composition is unknown; a relation to collagen is suggested by their sensitivity to bacterial collagenase [6], but they do not react with antibodies to any known collagen type.

Attempts to isolate and characterize the AF have been unsuccessful [7]. Using monoclonal techniques [8], we produced antibodies to the AF and have applied those antibodies to study EB.

## MATERIALS AND METHODS

### *Immunization and Cell Culture*

ME-180 cells, a human cervical carcinoma cell line, and the 8 M urea-dithreitol extract of human stratum corneum [9] were used as antigens for immunization. Murine monoclonal antibodies were produced using NS1/1-Ag-1 myeloma cells employing standard methods [6].

### *Antibody Testing and Characterization*

Antibodies initially were determined by radioimmunoassay using intact ME-180 cells as an antigen [10]. AF1 defines an antibody derived from the ME-180 immunization; AF2, an antibody from the stratum corneum immunization. (AF1 has been called DUX 5.2 previously [10].)

Immunoglobulin type was determined by Ouchterlony double-diffusion using immunoglobulin class-specific antisera (Miles Laboratories, Inc., Naperville, Illinois) and chromatographic behavior of the immunoglobulin on a staphylococcal protein A column (Sigma, St. Louis, Missouri) [11].

### *Immunofluorescence and Immunoglobulin Microscopy*

Immunofluorescence was performed by standard techniques [10] using as a second antibody 1:10 to 1:100 fluoresceinated rabbit anti-mouse IgG (Cappel Laboratories, Cochranville, Pennsylvania). Controls for immunofluorescent studies included concentrated myeloma supernatant and pure mouse $IgG_1$, isolated from mouse serum using a staphylococcal A column [11].

Immunoelectron microscopy was performed on O.C.T. (Miles Laboratories, Inc.) embedded tissues as previously detailed [10].

## RESULTS

Two murine monoclonal antibodies (AF1 and AF2), both $IgG_1$, kappa light chain, reacted with the human skin basement membrane zone and those human tissues known to contain AF (skin, cervix, esophagus, tongue, vagina, and buccal mucosa), but not with thyroid, kidney, lung, trachea, rectum, stomach, or ileum (Fig 1*A*). AF1 reacted with green macaque monkey skin, palm or chest, but not rat, mouse, guinea pig, or rabbit skin. Immunoelectron microscopy demonstrated localization of AF1 and AF2 to the area immediately subjacent to the lamina densa, although a somewhat different distribution of immunoreaction product was evident with the two antibodies. These patterns appeared to be independent of antibody concentration. AF1 was distributed in the lower (dermal) portion of the lamina densa, in the area immediately below the lamina densa, and on or near AF. The reaction product associated with AF1 tended to become confluent even with serial dilution. The immunoreactant produced by AF2 was more discrete and located in the area below the lamina densa associated with AF (Fig 2). To a lesser extent, the reaction product was present in the lamina densa. Rabbit antihuman fibronectin and the sera of a patient with acquired EB [12] did not interfere with AF1 binding to normal human skin.

### *Localization of Antibodies in Epidermolysis Bullosa*

Patients with various forms of inherited EB were studied to define further the antigenic specificity of the AF1 and AF2 (Table I), using immunofluorescent techniques. No reaction with either antibody was seen at the basement membrane zone of normal-appearing, unblistered skin or experimentally blistered skin of 11 patients with RDEB (Fig 1*B*). The never-blistered skin of a 33-h-old infant with RDEB did not react with AF1 or AF2. The unblistered skin of patients with junctional EB (5 patients), EB simplex (4 patients), and EB dystrophica dominant (Pasini type) (3 patients), all ultrastructurally confirmed, demonstrated basement membrane zone staining with both AF1 and AF2. In experimentally blistered skin of patients with junctional EB and EB simplex, both

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Abbreviations:
AF: anchoring fibril(s)
EB: epidermolysis bullosa
RDEB: recessive dystrophic epidermolysis bullosa

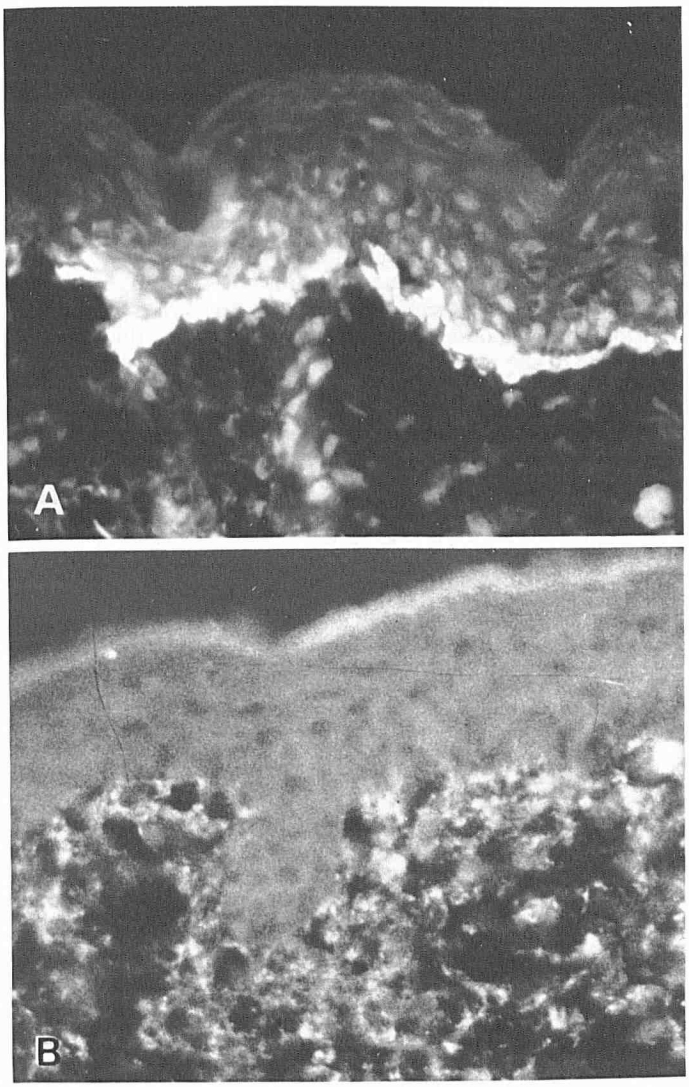


FIG 1. *A*, Normal human skin. The reaction of normal human skin with murine monoclonal antibody AF1. Staining was done as described in *Materials and Methods* with the addition of a methyl green counterstain which stains the nucleus. A thick subepidermal band of staining characterizes AF1 (original mag × 200). *B*, Nonblistered human skin from a patient with recessive dystrophic epidermolysis bullosa, stained with AF1 as described in *Materials and Methods*. No methyl green counterstain. No basement membrane zone staining (original mag × 200).

antibodies reacted positively and were localized to the dermal side of the induced epidermal-dermal separation. It was not possible to produce blisters experimentally in the dominant dystrophic patients.

Immunoelectron microscopy confirmed these observations. In RDEB no immunoreaction product was present below the lamina densa with either AF1 or AF2 (Fig 3). A paucity or absence of AF below the lamina densa was evident; whereas, in junctional EB, localization of AF1 and AF2 was seen (Fig 4) in the sub-basal lamina area in a distribution pattern similar to that seen in normal human skin controls (Fig 2).

## DISCUSSION

The monoclonal antibodies, AF1 and AF2, reacted with various tissues, including skin and esophagus, known to possess AF. The unblistered and experimentally blistered skin of patients with RDEB failed to react with both antibodies; whereas the skin of patients with junctional EB and EB simplex, which have a normal component of AF, did react. In severe generalized (gravis) RDEB epidermal-dermal separation occurred in the

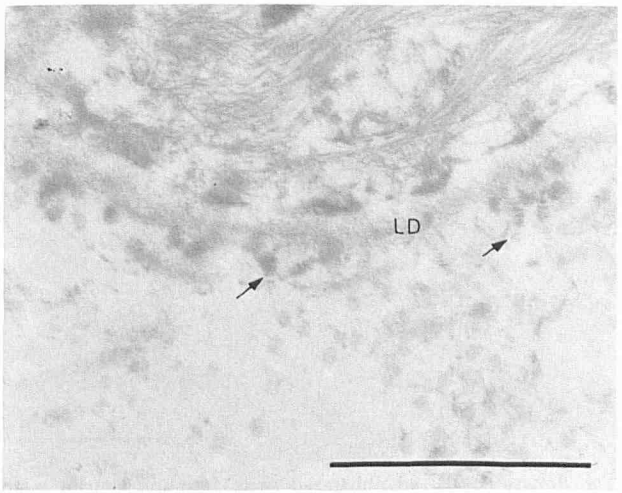


FIG 2. Normal human skin. Immunoelectron micrograph, using murine monoclonal antibody with junctional specificity, AF2, shows reaction product below the lamina densa (*LD*) in association with anchoring fibrils (*arrows*). Uranyl acetate and lead citrate stain. *Calibration bar* = 1 μm.

TABLE I. *Reactivity of junctional reacting monoclonal antibodies AF1 and AF2 in various forms of epidermolysis bullosa by immunofluorescence*

| Disease (No. of patients) | Unblistered skin | Experimentally blistered skin |
|---|---|---|
| | AF1 and AF2 | AF1 and AF2 |
| RDEB severe generalized gravis type (11) | — | – |
| Junctional EB (5) | + BMZ reaction | + Dermal side of separation |
| EB simplex (4) | + BMZ reaction | + Dermal side of separation |
| EB dystrophica dominant albopapuloid (Pasini) type (3) | + BMZ reaction | * |

BMZ = basement membrane zone; EB = epidermolysis bullosa; – = no reaction.
* Blisters could not be induced experimentally.

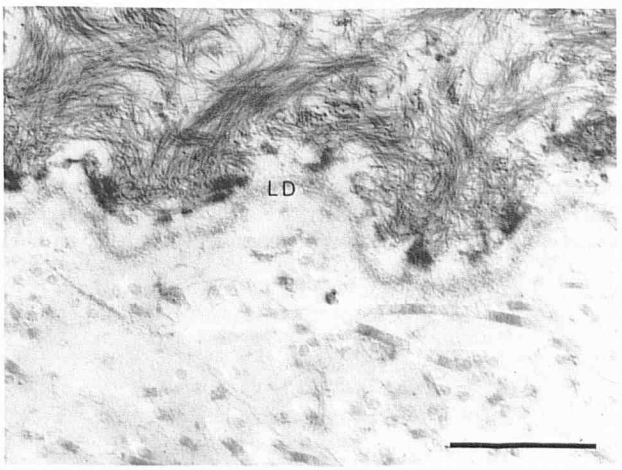


FIG 3. RDEB (severe generalized or gravis type). Unblistered skin. Immunoelectron micrograph using AF2 shows no reaction product below the lamina densa (*LD*). Note absence of AF below the lamina densa. Uranyl acetate and lead citrate stain. *Calibration bar* = 1 μm.

area below the lamina densa. AF are sparse or absent, even in the normal-appearing, unblistered skin of this disease [1,2]. On the other hand, junctional EB produces separation in the lamina rara and EB simplex cleaves within the cytoplasm of

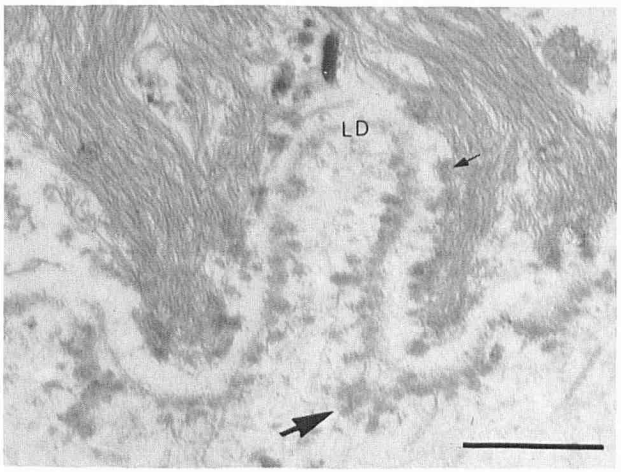


FIG 4. Junctional EB. Unblistered skin. Immunoelectron micrograph using AF2 shows reaction product below lamina densa (*LD*) in association with AF (*thick arrow*). Note small, densely stained, abnormal hemidesmosomes (*thin arrow*) which characterize junctional EB. Uranyl acetate and lead citrate stain. *Calibration bar* = 1 μm.

the epidermal basal cells [1]. In both of the latter diseases, AF are qualitatively and quantitatively normal.

Taken together with the immunoultrastructural localization of these antibodies in association with AF at the epidermal-dermal junction of skin, the findings indicate that the antigenic specificity of AF1 and AF2 is directed to AF, a portion of these structures, or a very closely associated antigenic molecule. In the Pasini form of EB, the reaction of AF1 and AF2 could not be distinguished from normal. Dominant dystrophic EB of the albopapuloid (Pasini) type presents morphologically abnormal AF in both normal-appearing and blister-prone skin areas which may or may not be decreased in number [4,5]. The component of the AF which may be altered in the Pasini disease is not altered antigenically as detected by AF1 and AF2.

We cannot determine with certainty at this time whether AF1 and AF2 have identical antigenic specificities, although they produce similar reactivity in EB skin and other tissues and similar localization below the lamina densa. However, the two antibodies have demonstrated consistently a somewhat different distribution pattern of immunoreaction product. This may indicate antigenic specificity to different portions of AF or associated antigens. Of the two antibodies, AF2 seems most definitely localized to AF on immunoelectron microscopy (Fig 2). The large size of the reaction product produced by peroxidase labeling, in comparison with the AF themselves, makes precise localization on the fibril somewhat uncertain.

On a practical note, both antibodies faithfully detect recessive EB with decreased or absent AF. Both the unblistered and blistered skin of RDEB were unreactive with AF1 and AF2. These antibodies could prove useful as an aid of diagnosis in EB, especially in situations where electron microscopy is not readily available (Fig 1*A,B*). Hintner et al have used pemphigoid antibodies and antibodies to type IV collagen and laminin to document the level of separation in the skin in various forms of EB [13]. Breathnach et al [14] have described a monoclonal antibody (KF-1) that reacts with a component of the lamina densa which is absent in dystrophic epidermolysis bullosa. The relationship of the component(s) identified by KF-1 and AF1 and AF2 remains to be determined.

AF1 and AF2 would be useful additions to such a panel of antibodies in the determination of specific types of EB. In addition, the antibodies may present a new tool for the objective evaluation of the response to treatment, and the antibodies can be used in the isolation and characterization of the AF.

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